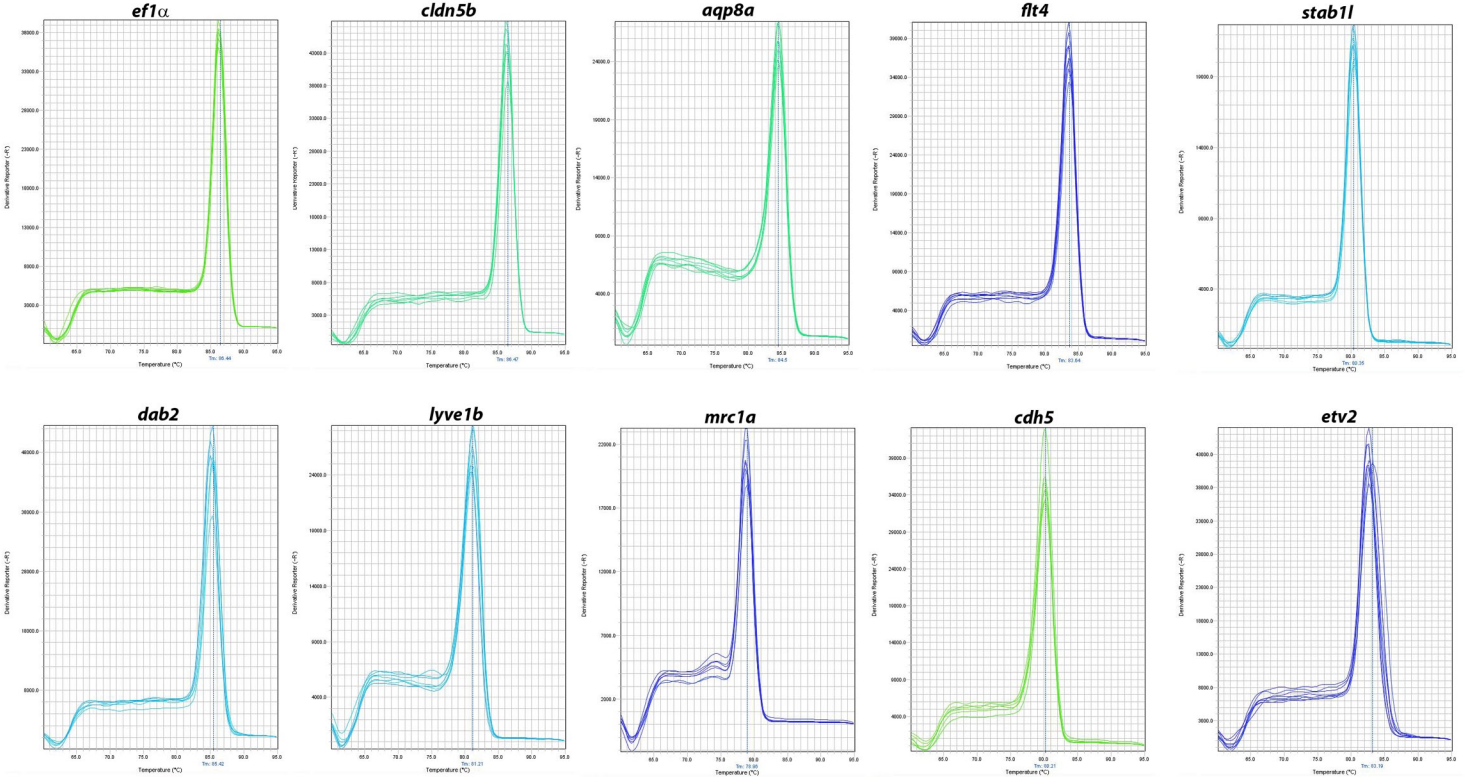
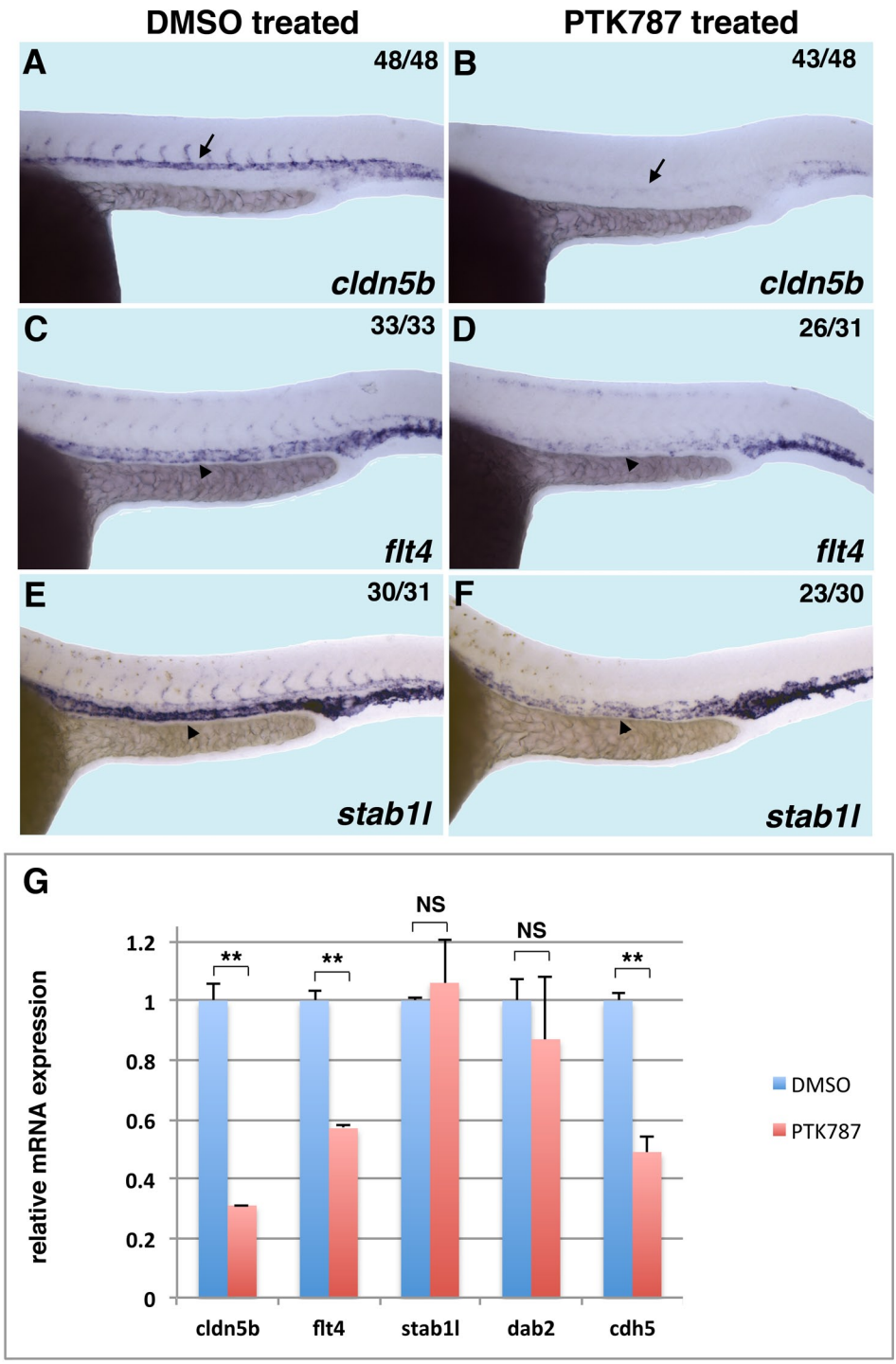
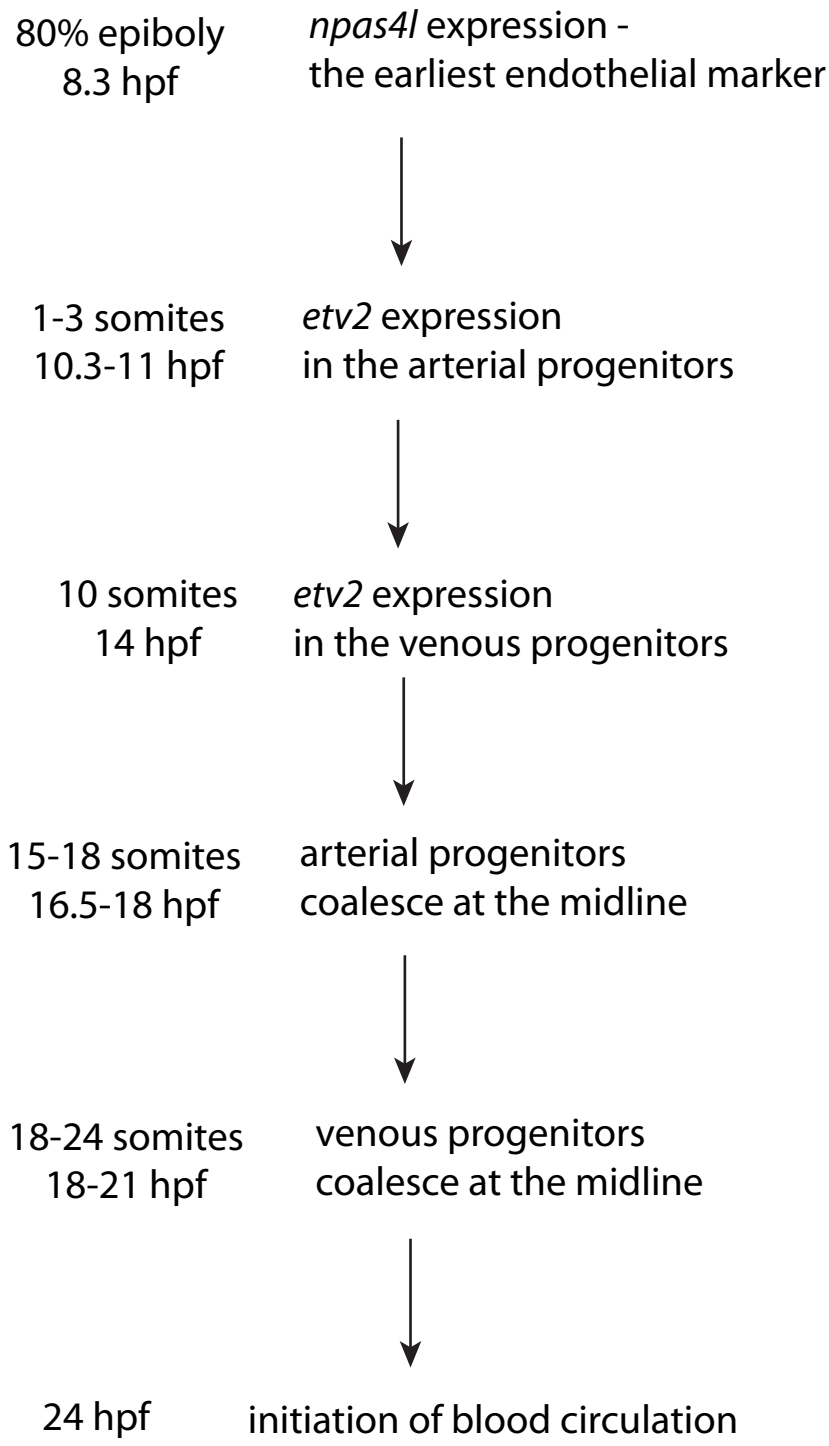


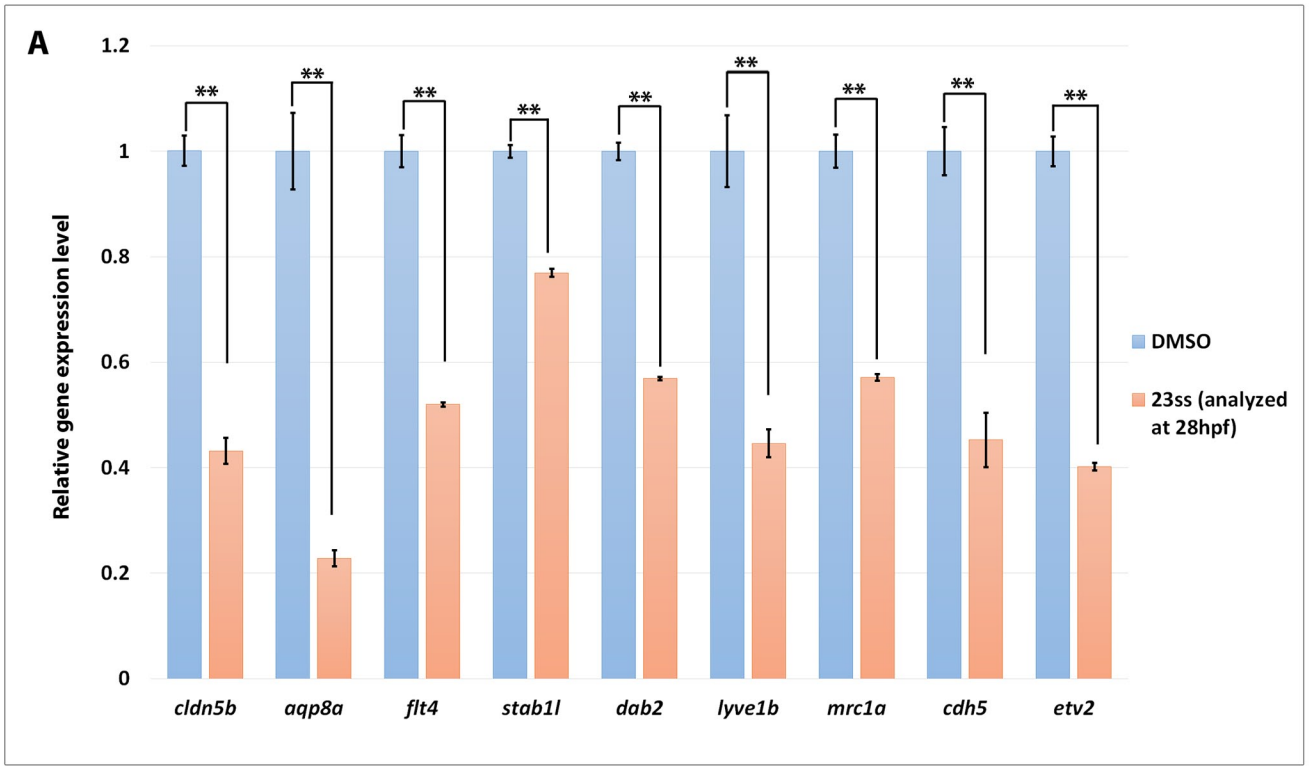

**Supplemental Figure S1. Melting curves of PCR products**. Single-peak melting curves for all analyzed genes indicate a single specific PCR product during qPCR analysis.

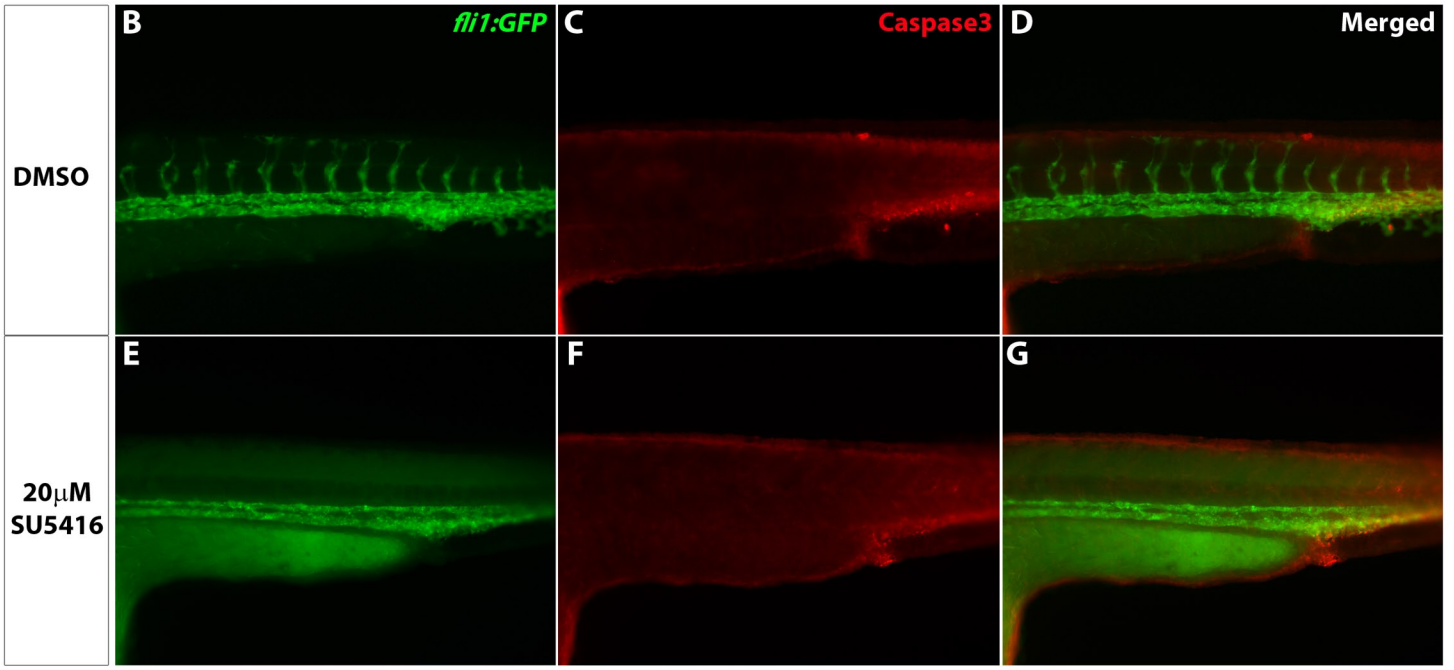



**Supplemental Figure S2**. **PTK787 chemical inhibition decreases arterial and venous marker expression and overall endothelial differentiation.** (A-F) In situ hybridization analysis for arterial *cldn5b* and venous *flt4* and *stab1l* expression at 24 hpf. Embryos were treated with 200 μM PTK787 beginning at the 50% epiboly stage. Note the downregulation of *cldn5b* and *flt4* expression in PTK787 treated embryos. Arrows indicate DA and arrowheads indicate PCV. Numbers of embryos showing the displayed phenotype are listed. (G) qPCR analysis of arterial (*cldn5b*), venous (*flt4, stab1l, dab2*) and pan-endothelial (*cdh5, etv2*) marker expression in PTK787 treated embryos at 24 hpf. Note that arterial marker, pan-endothelial marker and venous *flt4* expression is reduced, while *stab1l* and *dab2* expression is not changed significantly. ** denotes $p<0.01$; * denotes $p<0.05$; NS, not significant. ± SEM is shown.

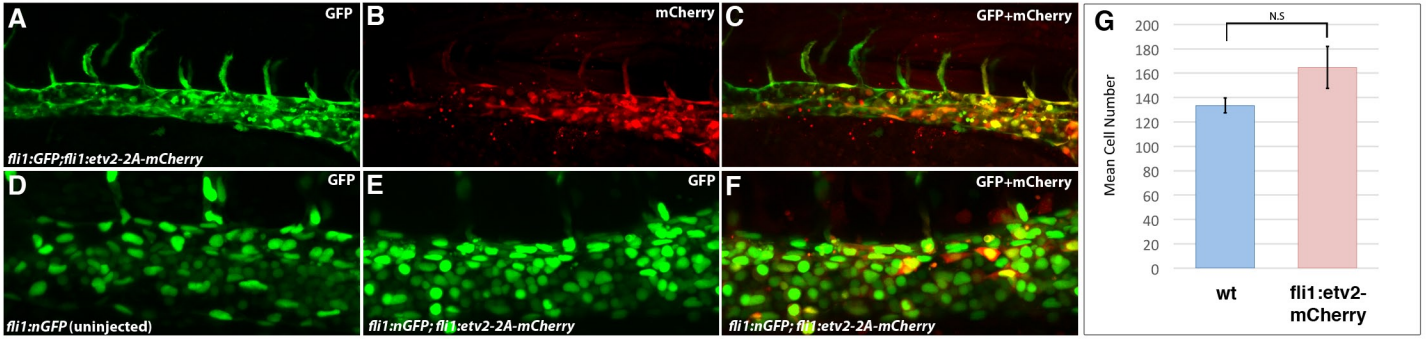



**Supplemental Figure S3. A timeline of the major events in zebrafish vasculogenesis.** Based on multiple publications including Kohli et al., 2013, and Reischauer et al., 2016.

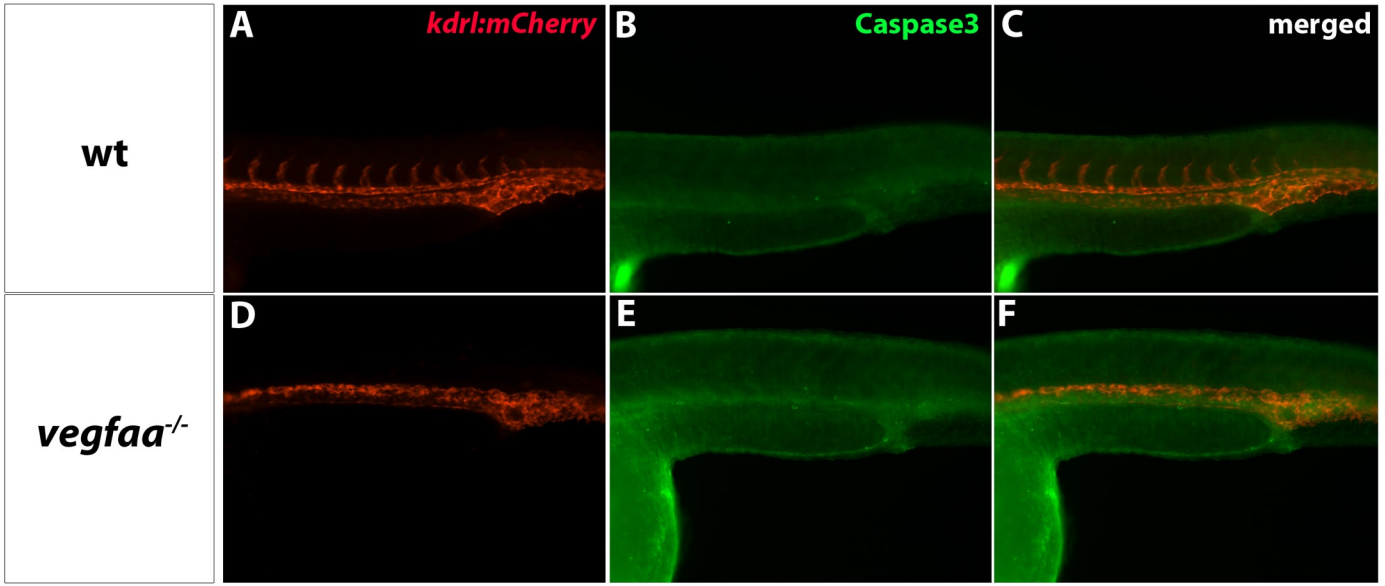



**Supplemental Figure S4. Analysis of marker expression and apoptosis in 28hpf embryos treated with SU5416 beginning at the 23-somite stage (20.5 hpf).** (A) qRT-PCR analysis shows a significant decrease of all vascular markers in SU5416-treated embryos compared to DMSO-treated controls at 28hpf, after commencing treatment at the 23-somite stage. (B-G) Double immunofluorescent staining of active Caspase3 and GFP at 28hpf in *fli1:GFP* embryos treated with either DMSO or 20μM SU5416 (beginning at the 23-somite stage). Note the absence of Caspase3 staining in the trunk region of both the DMSO- and SU5416-treated embryos (C,F), indicating a lack of apoptosis in the trunk vasculature.




**Supplemental Figure S5. Transient *fli1:etv2-2A-mCherry* expression in the vasculature does not affect the number of vascular endothelial cells.** (A-C) Confocal images of trunk vasculature of a *fli1:GFP* embryo injected with a *fli1:etv2-2A-mCherry* construct at 24 hpf. Note that mCherry is expressed in a mosaic fashion within the vasculature. (D-F) Confocal images of trunk axial vasculature in a control uninjected *fli1:nGFP* embryo (D) and a *fli1:etv2-2A-mCherry* injected embryo (E,F) at 24 hpf. (G) Quantification of vascular endothelial cell number in uninjected *fli1:nGFP* embryos (n=6) and *fli1:nGFP* embryos injected with *fli1:etv2-2A-mCherry* construct (n=8) at 24 hpf. No significant difference in endothelial cell number was observed between control and injected embryos. The cells were quantified in the axial vasculature in a two somite region using Imaris software.




**Supplemental Figure S6. *vegfaa* mutants do not display apoptosis in trunk vasculature at 24hpf.** (A-F) Double immunofluorescent staining of Caspase3 and mCherry in *vegfaa* mutants (in a *kdrl:mCherry* background). mCherry staining marks vasculature, while Caspase staining indicates cells undergoing apoptosis. *vegfaa* mutants did not show Caspase staining in the trunk vasculature (E) and were similar to their wild type siblings (B).

| Treatment/Condition | Gene | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *cldn5b* | *aqp8a* | *flt4* | *stab1l* | *dab2* | *lyve1b* | *mrc1a* | *cdh5* | *etv2* |
| **SU5416 - Concentration Gradient** | | | | | | | | | |
| 2.5μM | ↓↓ | ↓ | - | - | ↑ | - | ↓ | ↓ | - |
| 5μM | ↓↓ | ↓↓ | ↓ | ↓ | ↑ | - | ↓ | ↓ | ↓ |
| 10μM | ↓↓ | ↓↓ | ↓ | ↓ | ↓ | ↓↓ | ↓↓ | ↓ | ↓ |
| 20μM | ↓↓ | ↓ | ↓ | ↓ | ↓ | ↓↓ | ↓↓ | ↓↓ | ↓ |
| 20μM (analyzed at 15ss) | ↓ | ↓ | - | ↑↑ | - | ↓ | ↓ | ↓ | ↓ |
| | | | | | | | | | |
| **PTK787 (200μM)** | ↓↓ | | ↓ | - | - | | | ↓↓ | ↓ |
| | | | | | | | | | |
| **SU5416- Temporal treatment** | | | | | | | | | |
| 50% epiboly | ↓↓ | ↓↓ | ↓ | ↓ | - | ↓↓ | ↓↓ | ↓ | ↓ |
| 5-somite stage | ↓↓ | ↓↓ | ↓↓ | ↓↓ | ↓ | ↓↓ | ↓ | ↓↓ | ↓ |
| 15-somite stage | ↓↓ | ↓↓ | ↓ | - | ↓ | ↓ | ↓↓ | ↓↓ | ↓ |
| 20-somite stage | ↓ | ↓ | - | ↓ | - | ↓ | ↓ | ↓ | ↓ |
| 23-somite stage | ↓ | ↓ | ↓ | - | ↑↑ | ↓ | ↓ | - | ↓ |
| 23-somite stage (analyzed at 28hpf) | ↓↓ | ↓↓ | ↓ | ↓ | ↓ | ↓↓ | ↓ | ↓↓ | ↓↓ |
| | | | | | | | | | |
| **Vegfaa-/- mutants** | ↓ | ↓ | ↓ | ↓ | ↑ | ↑ | - | ↓ | ↓ |
| | | | | | | | | | |
| **Vegfaa RNA (overexpression)** | ↑↑ | ↑ | - | ↑ | ↓ | ↓↓ | ↓↓ | ↑↑ | ↑ |

**Supplemental Figure S7. Summary of the effect of all treatments, including mutants, on the expression levels of all analyzed genes.** Blue arrows indicate a decrease in expression, while red arrows indicate an increase in expression. Genes which decrease by more than 2-fold are represented by double blue arrows, while those that increase above 1.5-fold are represented by double red arrows. Dashes indicate that no significant change was observed.